A METHOD OF PRESERVING TYPHOID STOOLS FOR DELAYED EXAMINATION AND A COMPARATIVE STUDY OF THE EFFICACY OF EOSIN BRILLIANT-GREEN AGAR, EOSIN METHYLENE-BLUE AGAR, AND ENDO AGAR FOR THE ISOLATION OF TYPHOID BACILLI FROM STOOLS*

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Most laboratories in which examinations of typhoid stools are carried out, receive their specimens at various lengths of time after passage by the patient. In warm weather, stools, particularly if they are liquid, are apt to show, after 12 or more hours, a marked increase in the number of other bacteria as compared to that of the typhoid bacilli present. Since there is no satisfactory enrichment fluid for the typhoid bacillus, the success or failure in isolating typhoid bacilli from stools by our present methods depends primarily on the proportion of typhoid to other bacteria present; Ficker and Hofmann,[1] for example, estimated that 1:300 represents the lowest proportion of typhoid that still gives positive results. Hence, it seems highly desirable to preserve the original proportion of typhoid to other bacteria (that is, to prevent overgrowth of the typhoid by the latter) from the time that the stool is passed until it reaches the bacteriologist. The method to be described accomplishes this result, not only for periods of 8, 12, or 24 hours, but for a number of days; it therefore not only obviates the inaccuracies of diagnosis resulting from the usual delay in the arrival of specimens at the laboratory, but also permits of specimens' being sent from a whole state or country to a central station for examination.

Our first experiments in this direction were concerned with the use of hypertonic solutions of sodium chlorid. Preliminary tests as to the effect of the salt solution in various strengths on pure cultures of typhoid bacilli indicated that a 5% solution was the strongest that could be used without causing a rapid drop in the number of typhoid bacilli present. Sodium chlorid in this strength undoubtedly exerts

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[1] Arch. f. Hyg., 1904, 49, p. 229.

a marked inhibitory action on the growth of Bacillus coli. A series of typhoid stools were treated in this way, 5 c.c. of a suspension of each of the stools having been added to 5 c.c. of 10% sodium chlorid and another 5 c.c. of the stools to 5 c.c. of 0.6% sodium chlorid, as control. Attempts were made to isolate typhoid bacilli from both mixtures after they had stood at room temperature for 24 hours, 48 hours, 3 days, and 4 days respectively. In every instance typhoid bacilli could be recovered from the mixture containing 5% sodium chlorid after a longer interval than from the control mixture. Since we later found in glycerin a better medium for this purpose, it is unnecessary to tabulate these earlier results. Table 1 demonstrates the superiority of 25% glycerin over 5% sodium chlorid as a preservative of typhoid stools for delayed examination.

TABLE 1

COMPARISON OF 25% GLYCERIN AND 5% AND 0.6% SODIUM CHLORID AS PRESERVATIVES FOR TYPHOID BACILLI IN STOOLS

Period of Delay and Preservative Used	Methylene-blue Eosin Plates							
	1:100,000*		1:10,000		1:1000		1:100	
	Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others
At once	..	..	0	1	0	0	1	0
24 hr. .6% NaCl	..	..	0	2	1	5	4	36
24 hr. 5% NaCl	..	..	..	..	0	1	0	1
24 hr. 25% NaCl	..	..	..	..	0	1	1	1
48 hr. .6% NaCl	..	..	0	50	0	122	0	∞
48 hr. 5% NaCl	..	..	..	..	0	2	0	5
48 hr. 25% glycerin	..	..	..	..	0	0	0	0
72 hr. .6% NaCl	0	27	0	100	0	Num.	0	∞
72 hr. 5% NaCl	..	..	..	..	0	13	0	16
72 hr. 25% glycerin	..	..	..	..	0	0	0	0
4 da. .6% NaCl	0	70	0	Num.	0	∞	0	∞
4 da. 5% NaCl	..	..	..	..	0	21	0	85
4 da. 25% glycerin	..	..	..	..	0	0	0	7
5 da. .6% NaCl	0	Num.	0	Num.	0	∞	0	∞
5 da. 5% NaCl	..	..	0	5	0	31	0	Num.
5 da. 25% glycerin	..	..	.	..	0	47	0	25

Num. = numerous; ∞ = infinite numbers.
* Representing the dilution of the suspension of feces used for inoculation.

A stool of a typhoid-carrier, M. B., was rubbed up in 0.6% salt solution and filtered first through cotton and then through filter paper. After this treatment it could be assumed that the typhoid bacilli were uniformly distributed throughout the filtrate. Five cubic centimeters of the filtrate were added to 5 c.c. of 50% glycerin, another 5 c.c. to 10% sodium-chlorid solution, and finally 5 c.c. of the filtrate were added to 5 c.c. of 0.6% sodium chlorid as a control. The test tubes containing these mixtures were kept at room temperature in a dark closet. After intervals of 1, 2, 3, 4, and 5 days dilutions

of the 3 mixtures were prepared and 1 loop of each dilution was spread over one quadrant of an eosin methylene-blue plate and also on a quadrant of an eosin brilliant-green plate.

The number of typhoid colonies and the number of other colonies developing in each quadrant are recorded in the table. The dilution of the suspension of feces with which the quadrant was inoculated is indicated at the top of the column. For the identification of the typhoid bacilli, the appearance of the colonies and a macroscopic agglutination with immune serum on a slide were considered adequate since the stool was obtained from a person known to be a typhoid-carrier.

The eosin methylene-blue plate was recently devised by Holt-Harris and Teague[2] and the eosin brilliant-green plate by Teague and Clurman.[3] The same material was inoculated on both kinds of media in

TABLE 1—*Continued*

COMPARISON OF 25% GLYCERIN AND 5% AND 0.6% SODIUM CHLORID AS PRESERVATIVES FOR TYPHOID BACILLI IN STOOLS

Methylene-blue Eosin Plates				Brilliant-green Eosin Plates							
1:10		1:1		1:1000		1:100		1:10		1:1	
Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others
3	6	..	..	0	0	1	0	3	0	35	0
15	Num.	..	..	0	0	9	0	30	20	..	..
4	4	..	..	0	0	2	0	3	0	15	0
1	5	..	..	0	0	0	0	1	0	35	0
0	∞	..	..	0	110	0	Num.	0	∞	0	∞
1	Num.	2	∞	0	0	0	0	1	7	0	80
1	9	22	28	0	0	0	0	0	0	26	2
..	..	..	..	0	Num.	0	Num.	0	∞	..	..
0	Num.	0	∞	0	3	0	26	0	Num.	0	Num.
2	1	18	7	0	0	0	0	0	0	15	1
..	..	..	..	0	28	0	116	..	..	..	..
0	Num.	0	∞	0	7	0	57	0	Num.	0	∞
0	0	12	0	0	0	0	0	1	0	18	0
..	..	..	..	0	Num.	0	∞	..	..	..	..
0	∞	..	..	0	0	0	3	0	Num.	2	∞
2	5	12	73	0	0	1	0	2	0	16	0

order to obtain a more accurate picture of the action of the preservatives. With the eosin brilliant-green plate B. coli is inhibited to a great extent, so that one cannot judge from this plate alone of the relative numbers of typhoid bacilli and colon bacilli present at different intervals. The methylene-blue eosin plate indicates with fair accuracy

[2] Jour. Infect. Dis., 1916, 18, p. 596.
[3] Ibid., p. 647.

the proportion of typhoid bacilli to other bacteria present, but is not able to demonstrate the presence of a very small proportion of typhoid bacilli as does the eosin brilliant-green plate. The inoculation of the two kinds of media with the same material served the additional purpose of allowing us to make a comparative study of the mode of action of these two media.

It is seen from Table 1 that during the first 24 hours both the typhoid and the colon bacilli (we shall call the fecal bacteria colon

TABLE 2

RESULTS OF THE USE OF VARIOUS PERCENTAGES OF GLYCERIN AS PRESERVATIVE IN THE EXAMINATION OF TYPHOID STOOLS

Period of Delay and Percentage of Glycerin Used as a Preservative	Methylene-blue Eosin Plates					
	1:100,000*		1:10,000		1:1000	
	Typhoid	Others	Typhoid	Others	Typhoid	Others
At once	.....	.....	.....	.....	4	9
24 hr. .6% NaCl	.....	.....	0	0	4	56
24 hr. 20% glycerin	.....	.....	0	0	4	0
24 hr. 25% glycerin	.....	.....	0	1	5	1
24 hr. 30% glycerin	.....	.....	1	0	3	2
48 hr. .6% NaCl	0	10	0	54	0	Num.
48 hr. 20% glycerin	.....	.....	0	1	0	5
48 hr. 25% glycerin	.....	.....	0	0	0	3
48 hr. 30% glycerin	.....	.....	0	0	1	2
3 da. .6% NaCl	0	28	0	62	0	Num.
3 da. 20% glycerin	.....	.....	.....	.....	4	1
3 da. 25% glycerin	.....	.....	.....	.....	0	3
3 da. 30% glycerin	.....	.....	.....	.....	2	0
4 da. .6% NaCl	0	21	0	63	0	Num.
4 da. 20% glycerin	.....	.....	.....	.....	1	2
4 da. 25% glycerin	.....	.....	.....	.....	2	2
4 da. 30% glycerin	.....	.....	.....	.....	1	1

* Representing the dilution of the suspension of feces used for inoculation.
Num.= numerous; ∞ = infinite numbers.

bacilli, since no attempt was made to separate them into groups in these experiments) increase in number in 0.6% NaCl solution, the latter more rapidly than the former, however. In 5% NaCl solution and 25% glycerin both types of organisms remain constant as to number and proportion.

After 48 hours B. coli has increased enormously in 0.6% NaCl solution, so that typhoid bacilli can no longer be recovered even on the eosin brilliant-green plate. In 5% sodium-chlorid solution B. coli has increased, but to a much less extent than in the control mixture, while the typhoid bacilli have decreased in number. In 25% glycerin there is still very little change in the proportions or numbers of B. coli and typhoid bacilli present.

After 3 days there is a still further increase in B. coli in 5% sodium-chlorid solution and typhoid bacilli are no longer found. In the glycerin mixture B. coli has not increased and typhoid bacilli are reduced to about half the original number. After 4 days the same picture is presented.

After 5 days typhoid bacilli are still present in almost 50% of the original number in 25% glycerin, while colon bacilli have increased only slightly.

TABLE 2—*Continued*

RESULTS OF THE USE OF VARIOUS PERCENTAGES OF GLYCERIN AS PRESERVATIVE IN THE EXAMINATION OF TYPHOID STOOLS

Methylene-blue Eosin Plates		Brilliant-green Eosin Plates							
1:100		1:10,000		1:1000		1:100		1:10	
Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others
14	27	.....	.....	3	7	12	25	Num.	.....
6	Num.	0	2	1	12	4	76	10	Num.
17	24	.....	.....	0	0	12	2	Num.	.....
39	15	.....	.....	2	1	20	4	115	35
20	20	.....	.....	0	0	3	14	Num.	Num.
Few	Num.	.....	.....	3	10	2	58	4	Num.
13	26	.....	.....	2	1	15	0	68	30
10	23	.....	.....	2	0	8	1	95	30
7	5	.....	.....	0	0	6	2	50	50
0	∞	1	29	0	Num.	0	Num.	0	∞
15	15	.....	.....	0	0	13	5	51	11
12	16	.....	.....	0	0	8	1	65	15
3	17	.....	.....	0	0	5	3	40	15
0	∞	0	38	0	Num.	0	∞	.....	.....
10	15	.....	.....	1	0	6	0	79	8
7	11	.....	.....	.....	.....	2	0	67	2
4	9	.....	.....	0	0	3	0	27	0

Obviously, it will be impossible in practice to obtain constantly the same final percentage of glycerin with different specimens, since stools vary greatly as to the amount of water contained. Hence it was necessary to determine whether fairly wide variations in the percentage of glycerin would still yield satisfactory results.

For this purpose the stool of a typhoid patient (Z. S.) was suspended in 0.6% sodium-chlorid solution and filtered first through cotton and then through filter paper. Five cubic centimeters of filtrate were added to 5 c.c. of 40%, 50%, and 60% glycerin, severally, and to 0.6% sodium-chlorid solution as a control. The test tubes containing the mixtures were kept in the dark at room temperature. At 24-hour intervals dilutions in salt solution were prepared and 1 loop of each was spread over one quadrant of an eosin methylene-blue plate and on a quadrant of an eosin brilliant-green plate. The results of this experiment are recorded in Table 2.

TABLE 3

RESULTS OF THE USE OF DIFFERENT PERCENTAGES OF GLYCERIN AS PRESERVATIVE FOR TYPHOID STOOLS

Period of Delay and Percentage of Glycerin Used as Preservative	Methylene-blue Eosin Plates							
	1:100,000*		1:10,000		1:1000		1:100	
	Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others
Patient O. E. X-25.								
At once	.....	...	...	...	0	0	0	0
24 hr. .6% NaCl	.....	...	)	6	1	49	1	Num.
24 hr. 20% glycerin	.....	...	0	0	0	0	4	2
24 hr. 25% glycerin	.....	...	0	0	0	0	0	1
24 hr. 30% glycerin	.....	...	0	0	0	0	1	0
48 hr. .6% NaCl	0	12	0	41	1	Num.	—	∞
48 hr. 20% glycerin	.....	...	.....	...	0	0	3	16
48 hr. 25% glycerin	.....	...	.....	...	0	0	1	0
48 hr. 30% glycerin	.....	...	.....	...	0	0	2	0
3 da. .6% NaCl	0	3	0	30	—	Num.	—	∞
3 da. 20% glycerin	.....	...	.....	...	0	32	0	11
3 da. 25% glycerin	.....	...	.....	...	0	Num.	1	Num.
3 da. 30% glycerin	.....	...	.....	...	0	4	0	20
4 da. .6% NaCl	0	Num.	0	Num.	0	Num.	—	∞
4 da. 20% glycerin	.....	...	.....	...	1	11	1	Num.
4 da. 25% glycerin	.....	...	.....	...	0	0	0	5
4 da. 30% glycerin	.....	...	.....	...	0	0	0	1
5 da. .6% NaCl	0	Num.	—	Num.	—	∞	.....	...
5 da. 20% glycerin	.....	...	.....	...	0	Num.	5	∞
5 da. 25% glycerin	.....	...	.....	...	.....	...	0	Num.
5 da. 30% glycerin	.....	...	.....	...	.....	...	0	1
7 da. .6% NaCl	1	Num.	—	∞	—	∞	.....	...
7 da. 20% glycerin	.....	...	.....	...	—	Num.	1	∞
7 da. 25% glycerin	.....	...	.....	...	—	Num.	—	Num.
7 da. 30% glycerin	.....	...	.....	...	0	0	0	1
9 da. .6% NaCl	0	Num.	—	Num.	—	∞	.....	...
9 da. 20% glycerin	.....	...	.....	...	0	Num.	0	Num.
9 da. 25% glycerin	.....	...	.....	...	0	Num.	0	Num.
9 da. 30% glycerin	.....	...	.....	...	0	0	0	9
Patient M. W. X-25								
At once	.....	...	.....	...	0	68	—	Num.
24 hr. .6% NaCl	0	3	0	21	—	Num.	—	∞
24 hr. 20% glycerin	.....	...	0	11	0	75	—	Num.
24 hr. 25% glycerin	.....	...	0	10	0	60	1	Num.
24 hr. 30% glycerin	.....	...	0	10	0	34	1	Num.
48 hr. .6% NaCl	0	30	0	99	—	Num.	—	∞
48 hr. 20% glycerin	.....	...	0	12	0	57	—	Num.
48 hr. 25% glycerin	.....	...	0	8	0	68	1	Num.
48 hr. 30% glycerin	.....	...	0	6	0	24	—	Num.
3 da. .6% NaCl	0	90	—	Num.	.....	...	.....	...
3 da. 20% glycerin	0	3	0	28	—	Num.	—	Num.
3 da. 25% glycerin	0	1	0	9	0	42	—	Num.
3 da. 30% glycerin	0	0	0	2	1	21	—	Num.
4 da. .6% NaCl	0	34	—	Num.	—	∞	.....	...
4 da. 20% glycerin	0	0	0	13	0	76	—	Num.
4 da. 25% glycerin	0	0	0	7	0	57	—	Num.
4 da. 30% glycerin	0	0	0	4	0	12	—	Num.
5 da. .6% NaCl	—	Num.	—	Num.	—	∞	.....	...
5 da. 20% glycerin	0	6	0	62	—	Num.	—	∞
5 da. 25% glycerin	0	18	0	35	—	Num.	—	∞
5 da. 30% glycerin	0	4	0	11	0	56	—	Num.
9 da. .6% NaCl	—	Num.	—	∞	—	∞	.....	...
9 da. 20% glycerin	—	5	—	11	—	Num.	—	∞
9 da. 25% glycerin	0	1	0	15	—	Num.	—	∞
9 da. 30% glycerin	0	0	0	2	0	25	—	Num.

TABLE 3—*Continued*

RESULTS OF THE USE OF DIFFERENT PERCENTAGES OF GLYCERIN AS PRESERVATIVE FOR TYPHOID STOOLS

Methylene-blue Eosin Plates				Brilliant-green Eosin Plates							
1:10		1:1		1:1000		1:100		1:10		1:1	
Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others
10	15	—	160	0	0	4	0	12	0	86	7
—	∞	.....	...	1	1	10	12	—	Num.	—	Num.
10	22	.....	...	0	0	3	0	9	0	87	6
12	2	.....	...	0	0	0	0	3	0	46	3
7	7	.....	...	0	0	1	0	9	0	28	1
.....	...	.....	...	0	40	—	Num.	—	Num.	—	∞
11	69	—	Num.	0	0	2	1	17	10	Num.	Num.
2	12	29	44	0	0	0	0	2	3	25	7
2	3	20	31	0	0	0	0	4	0	30	2
.....	...	.....	...	1	51	—	Num.	—	∞	.....	....
21	Num.	—	Num.	0	6	3	1	17	2	Num.	Num.
3	Num.	21	Num.	0	0	0	0	3	0	15	1
3	Num.	7	Num.	0	0	0	0	1	0	8	0
.....	...	.....	...	0	53	—	Num.	.....	...	.....	....
0	Num.	3	Num.	0	0	1	2	10	4	Num.	Num.
2	Num.	7	Num.	0	0	0	0	0	0	3	1
0	1	13	14	0	0	1	0	0	0	8	0
.....	...	.....	...	0	Num.	—	∞	.....	...	.....	....
—	∞	—	∞	.....	...	1	10	7	76	—	Num.
2	Num.	4	∞	.....	...	0	0	0	0	5	9
2	7	27	17	.....	...	0	0	0	0	9	1
.....	...	.....	...	0	47	.....	...	.....	...	.....	....
—	∞	—	∞	0	2	2	15	—	88	—	Num.
—	∞	—	∞	0	0	0	0	0	1	5	4
0	0	6	Num.	0	0	0	0	0	0	11	0
.....	...	.....	...	0	34	.....	...	.....	...	.....	....
—	∞	—	∞	0	0	0	0	0	12	0	35
—	∞	—	∞	0	0	0	0	0	0	0	2
5	9	—	Num.	0	0	0	0	0	0	2	0
—	∞	—	∞	0	0	0	0	12	2	91	9
.....	...	.....	...	0	0	0	0	0	7	7	Num.
—	∞	.....	...	0	0	2	0	10	2	Num.	—
—	∞	.....	...	0	0	2	1	9	31	Num.	—
—	∞	.....	...	0	0	0	1	5	9	Num.	—
.....	...	.....	...	0	Num.	2	∞	—	∞	.....	....
—	∞	.....	...	0	0	1	1	8	15	Num.	—
—	∞	.....	...	0	3	1	3	12	17	Num.	—
—	∞	.....	...	0	0	1	1	3	17	Num.	—
.....	...	.....	...	0	0	0	Num.	0	Num.	.....	....
.....	...	.....	...	0	0	1	0	17	1	61	2
.....	...	.....	...	0	0	2	0	5	1	68	7
.....	...	.....	...	0	0	0	1	5	1	42	2
.....	...	.....	...	0	∞	1	∞	6	∞	—	∞
....	...	.....	...	0	0	2	0	5	0	56	13
.....	...	.....	...	0	0	2	0	8	1	62	3
.....	...	.....	...	0	0	1	0	3	0	59	0
.....	...	.....	...	1	∞	4	∞	—	∞	—	∞
.....	...	.....	...	2	1	3	0	38	3	Num.	20
.....	...	.....	...	0	0	1	0	25	5	Num.	—
.....	...	.....	...	0	0	1	0	11	1	Num.	10
.....	...	.....	...	—	∞	—	∞	.....	...	.....	....
.....	...	.....	...	0	0	0	0	12	0	Num.	—
.....	...	.....	...	0	0	0	1	12	2	45	3
.....	...	.....	...	0	0	0	0	2	1	40	6

TABLE 3—*Continued*

RESULTS OF THE USE OF DIFFERENT PERCENTAGES OF GLYCERIN AS PRESERVATIVE FOR TYPHOID STOOLS

Period of Delay and Percentage of Glycerin Used as Preservative	Methylene-blue Eosin Plates							
	1:100,000*		1:10,000		1:1000		1:100	
	Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others
Patient M. B. X-25								
At once	.....	...	.....	...	0	1	—	Num.
24 hr. .6% NaCl	.....	...	0	6	0	30	1	Num.
24 hr. 20% glycerin	.....	...	.....	...	0	0	0	8
24 hr. 25% glycerin	.....	...	.....	...	0	0	0	2
24 hr. 30% glycerin	.....	...	.....	...	0	0	0	4
48 hr. .6% NaCl	.....	...	0	35	0	110	—	Num.
48 hr. 20% glycerin	.....	...	.....	...	0	25	—	Num.
48 hr. 25% glycerin	.....	...	.....	...	0	0	0	13
48 hr. 30% glycerin	.....	...	.....	...	0	5	0	19
3 da. .6% NaCl	0	13	0	28	—	Num.	—	∞
3 da. 20% glycerin	.....	...	0	Num.	0	Num.	0	Num.
3 da. 25% glycerin	.....	...	.....	...	0	Num.	0	Num.
3 da. 30% glycerin	.....	...	.....	...	0	0	0	Few
4 da. .6% NaCl	0	30	0	50	—	Num.	—	∞
4 da. 20% glycerin	.....	...	0	2	0	Num.	0	Num.
4 da. 25% glycerin	.....	...	.....	...	0	0	0	6
4 da. 30% glycerin	.....	...	.....	...	0	0	0	0
5 da. .6% NaCl	—	Num.	—	Num.	—	∞	—	∞
5 da. 20% glycerin	.....	...	.....	...	.....	...	0	Num.
5 da. 25% glycerin	.....	...	.....	...	.....	...	0	Num.
5 da. 30% glycerin	.....	...	.....	...	.....	...	0	10
7 da. .6% NaCl	0	63	—	Num.	—	∞	.....	...
7 da. 20% glycerin	.....	...	.....	...	0	Num.	0	∞
7 da. 25% glycerin	.....	...	.....	...	0	Num.	0	Num.
7 da. 30% glycerin	.....	...	.....	...	0	0	0	6
9 da. .6% NaCl	—	Num.	—	∞	—	∞	.....	...
9 da. 20% glycerin	.....	...	.....	...	0	Num.	0	Num.
9 da. 25% glycerin	.....	...	.....	...	0	Num.	0	Num.
9 da. 30% glycerin	.....	...	.....	...	0	4	0	6

* Representing the dilution of the suspension of feces used for inoculation.
Num. = numerous; — = too many colonies to make the determination of typhoid bacilli possible; ∞ = infinite numbers.

Table 2 indicates that final concentrations of 20% and of 30% glycerin preserve the typhoid bacilli in the stool and prevent the growth of colon bacilli for a period of 4 days as satisfactorily as 25% glycerin.

Table 3 shows the results of similar experiments with other stools. The feces were filtered through a loose layer of cotton only, in order to approximate the actual conditions of practice more nearly, and the examinations covered longer periods. A dash in the table indicates that the colonies in that quadrant were so numerous and consequently so small that it was impossible to determine whether or not typhoid bacilli were present or, if present, it was impossible to estimate their number accurately.

TABLE 3—*Continued*

RESULTS OF THE USE OF DIFFERENT PERCENTAGES OF GLYCERIN AS PRESERVATIVE FOR TYPHOID STOOLS

Methylene-blue Eosin Plates				Brilliant-green Eosin Plates							
1:10		1:1		1:1000		1:100		1:10		1:1	
Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others
2	Num.	5	Num.	1	0	0	0	0	0	6	2
—	∞	.....	...	0	1	1	7	25	52	—	Num.
—	Num.	6	Num.	0	0	0	0	1	0	8	2
4	Num.	6	Num.	0	0	0	0	1	1	16	1
—	Num.	6	Num.	0	0	0	0	6	0	4	1
—	∞	.....	...	0	22	—	Num.	—	∞	—	∞
1	Num.	4	∞	0	1	0	0	1	2	4	74
0	Num.	2	Num.	0	0	0	0	0	0	2	2
—	Num.	—	Num.	0	0	0	0	2	0	4	1
.....	...	.....	...	2	14	—	Num.	—	∞	.....	....
0	Num.	.....	...	0	0	0	0	0	0	6	10
0	Num.	4	Num.	0	0	0	0	0	0	0	1
0	Num.	2	Num.	0	0	0	0	0	0	2	0
.....	...	.....	...	0	6	0	31	—	Num.	—	∞
—	Num.	.....	...	0	0	0	0	1	0	6	20
0	Num.	1	Num.	0	0	0	0	0	0	2	1
1	Num.	8	Num.	0	0	0	0	0	0	3	1
.....	...	.....	...	—	Num.	—	∞	—	∞	—	∞
2	Num.	5	∞	.....	...	0	0	0	4	7	47
0	∞	5	∞	.....	...	0	0	0	0	5	1
1	Num.	8	Num.	.....	...	0	0	0	0	14	4
.....	...	.....	...	0	Num.	—	∞	.....	...	.....	....
3	∞	2	∞	0	0	0	1	0	17	—	Num.
0	∞	2	∞	0	0	0	1	0	2	8	0
0	Num.	1	Num	0	0	0	0	0	0	9	10
.....	...	.....	...	—	Num.	.....	...	.....	...	.....	....
0	Num.	0	∞	0	0	0	1	1	1	11	15
0	∞.	4	∞	0	0	0	0	0	0	5	1
0	Num.	2	Num	0	0	0	0	0	0	3	0

It is seen from Table 3 that B. coli is not inhibited so well in 20% glycerin as in the other two mixtures, and typhoid bacilli decrease in number a little more rapidly in 30% glycerin than in the others, but all three percentages yield eminently satisfactory results. By furnishing 30% glycerin in sterile bottles to which some of the stool is to be added it will be easy to obtain in practice final concentrations lying between 20% and 30%.

In order to determine more accurately the total number of viable organisms in the glycerin mixture after different intervals of time it was decided to plate in plain nutrient agar 0.5 c.c. of appropriate dilutions and record the number of colonies.

A mixture of 6 typhoid stools and 6 normal stools was used to insure a great variety of fecal bacteria. The stools were emulsified in salt solution, thoroughly mixed together, and filtered through a thin layer of cotton. Five

TABLE 4

RESULTS OF THE USE OF GLYCERIN AS A PRESERVATIVE IN THE EXAMINATION OF TYPHOID STOOLS

Period of Delay and Percentage of Glycerin Used as a Preservative	Number of Colonies per 0.5 c.c.	Endo Plates							
		1:10,000,000*		1:1,000,000		1:100,000		1:10,000	
		Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others
At once	2,000,000	.....	.....	.....	.....	.....	.....	.....	.....
24 hr. 25% glycerin	1,800,000	.....	.....	.....	.....	.....	.....	0	8
24 hr. .6% NaCl	65,000,000	.....	.....	.....	.....	0	Num.	0	Num.
48 hr. 25% glycerin	1,500,000	.....	.....	.....	.....	.....	.....	0	3
48 hr. .6% NaCl	758,000,000	.....	.....	.....	.....	0	Num.	0	Num.
3 da. 25% glycerin	800,000	.....	.....	.....	.....	.....	.....	.....	.....
3 da. .6% NaCl	900,000,000	.....	.....	0	26	0	Num.	—	∞
4 da. 25% glycerin	350,000	.....	.....	.....	.....	.....	.....	.....	.....
4 da. .6% NaCl	15,000,000,000	.....	.....	0	Num.	—	∞	—	∞
7 da. 25% glycerin	.....	.....	.....	.....	.....	.....	.....	.....	.....
7 da. .6% NaCl	.....	—	∞	—	∞	.....	.....	.....	.....

* Representing the dilution of the suspension of feces used for inoculation.
Num. = numerous; ∞ = infinite numbers; — = too many colonies to make and accurate determination of typhoid bacilli possible.

TABLE 4—*Continued*

RESULTS OF THE USE OF GLYCERIN AS A PRESERVATIVE IN THE EXAMINATION OF TYPHOID STOOLS

Period of Delay and Percentage of Glycerin Used as a Preservative	Number of Colonies per 0.5 c.c.	Methylene-blue Eosin Plates					
		1:10,000		1:1000		1:100	
		Typhoid	Others	Typhoid	Others	Typhoid	Others
At once	2,000,000	.....	.....	0	14	2	96
24 hr. 25% glycerin	1,800,000	0	0	1	10	0	65
24 hr. .6% NaCl	65,000,000	0	35	0	Num.	—	∞
48 hr. 25% glycerin	1,500,000	0	1	0	6	1	36
48 hr. .6% NaCl	758,000,000	0	Num.	—	∞	—	∞
3 da. 25% glycerin	800,000	.....	.....	0	3	0	31
3 da. .6% NaCl	900,000,000	—	∞	—	∞	.....	.....
4 da. 25% glycerin	350,000	.....	.....	0	1	1	25
4 da. .6% NaCl	15,000,000,000	—	∞	—	∞	.....	.....
7 da. 25% glycerin	.....	.....	.....	0	2	0	4
7 da. .6% NaCl	.....	—	∞	—	∞	.....	.....

Num.= numerous; ∞ = infinite numbers; — = too many colonies to make and accurate determination of typhoid bacilli possible.

TABLE 4—*Continued*

RESULTS OF THE USE OF GLYCERIN AS A PRESERVATIVE IN THE EXAMINATION OF TYPHOID STOOLS

Endo Plates								Methylene-blue Eosin Plates					
1:1000		1:100		1:10		1:1		1:10,000,000		1:1,000,000		1:100,000	
Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others
2	24	0	140	—	Num.	.....	.....	.....	.....	.....	.....	.....	.....
1	Num.	0	Num.	—	Num.	.....	.....	.....	.....	.....	.....	.....	.....
0	Num.	—	∞	.....	.....	.....	.....	.....	.....	.....	.....	0	3
0	6	0	74	—	Num.	.....	.....	.....	.....	.....	.....	.....	.....
—	Num.	—	∞	.....	.....	.....	.....	.....	.....	.....	.....	0	54
0	10	1	51	—	Num.	—	∞	.....	.....	.....	.....	.....	.....
—	∞	.....	.....	.....	.....	.....	.....	.....	.....	0	14	0	Num.
0	7	0	45	0	Num.	—	∞	.....	.....	.....	.....	.....	.....
—	∞	.....	.....	.....	....	.....	.....	.....	.....	0	15	0	Num.
0	2	0	26	—	Num.	—	Num.	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	—	Num.	—	∞	—	∞

TABLE 4—*Continued*

RESULTS OF THE USE OF GLYCERIN AS A PRESERVATIVE IN THE EXAMINATION OF TYPHOID STOOLS

Methylene-blue Eosin Plates				Brilliant-green Plates									
1:10		1:1		1:10,000		1:1000		1:100		1:10		1:1	
Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others
—	Num.	.....	.....	.....	.....	0	0	5	0	21	6	Num.	—
—	Num.	.....	.....	.....	.....	0	0	2	0	11	7	Num.	—
.....	.....	.....	.....	0	1	0	56	0	Num.	—	∞	.....	.....
—	Num.	.....	.....	.....	.....	0	0	0	0	6	2	Num.	—
.....	.....	.....	.....	0	12	0	48	—	Num.	—	∞	.....	.....
												2 loops	
4	Num.	—	∞	.....	.....	.....	.....	.....	.....	6	7	90	25
.....	.....	.....	.....	0	9	—	Num.	—	∞	.....	.....	.....	.....
												2 loops	
—	Num.	—	∞	.....	.....	.....	.....	.....	.....	5	1	Num.	—
.....	.....	.....	.....	0	Num.	—	Num.	—	∞	.....	.....	.....	.....
0	Num.	—	∞	.....	.....	.....	.....	.....	.....	10	10	Num.	—
.....	.....	.....	.....	—	Num.	—	∞	.....	.....	.....	.....	.....	.....

cubic centimeters of the filtrate were added to 5 c.c. of 50% glycerin in 0.6% sodium-chlorid solution and another 5 c.c. to 5 c.c. of 0.6% sodium-chlorid solution alone. Dilutions were prepared at intervals and 1 loop of each was inoculated on a quadrant of an Endo, an eosin methylene-blue, and an eosin brilliant-green plate. On the 3rd, 4th, and 7th days 2 loops instead of 1 were inoculated on the eosin brilliant-green plate. At the same time 0.5 c.c. amounts of each of appropriate dilutions of the glycerin mixture and of the 0.6% sodium-chlorid-solution control were plated in ordinary nutrient agar. The results of this experiment are recorded in Table 4.

It is seen in Table 4 that in the glycerin mixture there was a gradual decrease in the number of viable bacteria from day to day, so that after 3 days less than half the original number were present; in the con-

TABLE 5

RESULTS OF THE USE OF GLYCERIN PLUS BROTH AS A PRESERVATIVE IN THE EXAMINATION OF TYPHOID STOOLS

Period of Delay and Percentage of Glycerin Used as Preservative	Endo Plates			
	1:1000*		1:100	
	Typhoid	Others	Typhoid	Others
Patient A. P. VIII-4				
At once	4	23	10	80
24 hr. 25% glycerin and 4% NaCl	2	8	1	24
24 hr. 20% glycerin	0	16	4	86
24 hr. 25% glycerin	0	5	10	30
24 hr. 25% glycerin and 4% NaCl in broth	0	1	0	35
24 hr. 25% glycerin in broth	1	2	8	42
48 hr. 25% glycerin and 4% NaCl	0	1	5	13
48 hr. 20% glycerin	0	8	4	101
48 hr. 25% glycerin	1	0	17	12
48 hr. 25% glycerin and 4% NaCl in broth	0	1	4	23
48 hr. 25% glycerin in broth	0	4	4	39
3 da. 25% glycerin and 4% NaCl	0	0	2	16
3 da. 20% glycerin	0	41	—	Num.
3 da. 25% glycerin	0	2	4	8
3 da. 25% glycerin and 4% NaCl in broth	0	5	3	11
3 da. 25% glycerin in broth	0	21	0	45
5 da. 25% glycerin and 4% NaCl	0	5	0	29
5 da. 20% glycerin	—	Num.	—	∞
5 da. 25% glycerin	1	0	1	2
5 da. 25% glycerin and 4% NaCl in broth	0	9	2	13
5 da. 25% glycerin in broth	1	11	12	33

* Representing dilution of the suspension of feces used for inoculation.
Num. = numerous; ∞ = infinite numbers; — = too many colonies to make an accurate determination of the typhoid bacilli.

trol mixture the bacteria increased from 2 million to 65 million during the first 24 hours and the typhoid bacilli were during this short interval so overgrown by the other bacteria that they were not recovered from any of the plates. From the glycerin mixture typhoid bacilli were readily recovered on the 7th day.

Altho the proportion of typhoid to colon bacilli in 25% glycerin remains constant or is altered in favor of the typhoid bacilli, yet there is a gradual decrease in the absolute number of typhoid bacilli present. It was thought that this decrease might perhaps be obviated by adding a small amount of broth to the glycerin.

The stool of a typhoid patient (A. P.) was rubbed up in salt solution and filtered through a thin layer of cotton. Fifty-percent glycerin was prepared in ordinary nutrient broth and 5 c.c. of this were mixed with 5 c.c. of the filtered feces. This mixture was compared with 20% and 25% glycerin in 0.6% NaCl solution and with 25% glycerin in combination with 4% sodium-chlorid solution. The results are shown in Table 5.

TABLE 5—*Continued*

RESULTS OF THE USE OF GLYCERIN PLUS BROTH AS A PRESERVATIVE IN THE EXAMINATION OF TYPHOID STOOLS

Endo Plates		Brilliant-green Eosin Plates					
1:10		1:1000		1:100		1:10	
Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others
—	Num.	0	0	9	12	81	6
—	Num.	1	0	6	1	38	2
—	Num.	3	0	18	3	Num.	—
—	Num.	1	0	18	0	Num.	—
—	∞	1	0	8	0	34	3
—	Num.	0	0	7	1	57	8
—	Num.	0	0	2	0	21	1
—	Num.	0	0	0	4	50	31
—	Num.	0	0	6	0	80	0
—	Num.	0	0	0	0	20	0
—	Num.	0	0	3	0	58	2
—	Num.	0	0	1	0	3	0
—	∞	0	3	2	7	Few	Num.
—	Num.	0	0	3	0	32	0
—	Num.	1	0	1	0	12	0
—	Num.	1	0	3	2	48	2
6	57	0	0	0	0	17	0
—	∞	0	5	0	60	—	Num.
20	36	1	0	3	0	19	0
11	67	0	0	2	0	21	0
—	Num.	1	0	7	0	47	0

There seems to be no practical advantage gained by adding sodium chlorid or broth to 25% glycerin. Table 5 shows that there is better inhibition of B. coli in 25% glycerin than in 20% and that the addition of broth to 25% glycerin impairs the inhibition of B. coli slightly.

In the previous experiments, all of which show that typhoid bacilli can be recovered from glycerin mixtures long after it is impossible to obtain them from the salt-solution control, the stools were carefully

TABLE 6

RESULTS OF THE USE OF GLYCERIN AS PRESERVATIVE IN ROUTINE EXAMINATION OF TYPHOID STOOLS

Period of Delay and Percentage of Glycerin Used as a Preservative	Methylene-blue Eosin Plates					
	1:10,000		1:1000		1:100	
	Typhoid	Others	Typhoid	Others	Typhoid	Others
Patient E. O. X-4						
At once	.....	.....	.....	.....	.....	.....
24 hr. 30% glycerin	.....	.....	.....	.....	.....	.....
48 hr. 30% glycerin	.....	.....	.....	.....	.....	.....
48 hr. Control stool	.....	.....	.....	.....	.....	.....
3 da. 30% glycerin	1	8	6	15	—	Num.
3 da. Control stool	4	115	—	Num.	—	∞
7 da. 30% glycerin	0	4	1	Num.	4	Num.
7 da. Control stool	0	Num.	—	Num.	—	∞
10 da. 30% glycerin	.....	.....	.....	.....	.....	.....
10 da. Control stool	.....	.....	.....	.....	.....	.....
15 da. 30% glycerin	.....	.....	.....	.....	.....	.....
15 da. Control stool	.....	.....	.....	.....	.....	.....
Patient M. W. X-4						
At once	.....	.....	.....	.....	.....	.....
48 hr. 30% glycerin	.....	.....	.....	.....	.....	.....
48 hr. Control stool	.....	.....	.....	.....	.....	.....
10 da. 30% glycerin	0	Num.	0	Num.	0	Num.
10 da. Control stool	0	Num.	0	Num.	—	∞
15 da. 30% glycerin	.....	.....	.....	.....	.....	.....
15 da. Control stool	.....	.....	.....	.....	.....	.....
Patient J. L. X-4						
At once	.....	.....	.....	.....	.....	.....
48 hr. 30% glycerin	.....	.....	.....	.....	.....	.....
48 hr. Control stool	.....	.....	.....	.....	.....	.....
3 da. 30% glycerin	4	4	12	17	—	95
7 da. 30% glycerin	1	7	0	7	5	31
7 da. Control stool	0	Num.	—	Num.	—	∞
10 da. 30% glycerin	.....	.....	0	2	0	24
15 da. 30% glycerin	.....	.....	.....	.....	.....	.....
15 da. Control stool	.....	.....	.....	.....	.....	.....
22 da. 30% glycerin	.....	.....	0	Num.	—	∞
Patient M. B. X-4						
At once	.....	.....	.....	.....	.....	.....
48 hr. 30% glycerin	.....	.....	.....	.....	.....	.....
48 hr. Control stool	.....	.....	.....	.....	.....	.....
3 da. 30% glycerin	3	56	8	Num.	—	∞
7 da. 30% glycerin	0	22	5	21	4	Num.
7 da. Control stool	0	∞	—	∞	—	∞
10 da. 30% glycerin	0	4	0	10	0	Num.
10 da. Control stool	.....	.....	.....	.....	.....	.....
15 da. 30% glycerin	.....	.....	.....	.....	.....	.....
15 da. Control stool	.....	.....	.....	.....	.....	.....

TABLE 6—*Continued*

RESULTS OF THE USE OF GLYCERIN AS PRESERVATIVE IN ROUTINE EXAMINATION OF TYPHOID STOOLS

Methylene-blue Eosin Plates		Brilliant-green Eosin Plates							
1:10		1:1000		1:100		1:10		1:1	
Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others
.....	.....	0	0	1	0	17	7	Num.	—
.....	.....	.....	.....	0	2	1	15	—	Num.
.....	.....	0	0	0	1	2	5	12	Num.
.....	.....	0	1	0	1	0	33	0	Num.
—	Num.	.....	.....	.....	.....	.....	.....	.....	.....
—	∞	.....	.....	.....	.....	.....	.....	.....	.....
1	Num.	0	2	6	19	9	Num.	—	∞
—	∞	0	Num.	0	Num.	—	∞	—	∞
.....	.....	0	0	0	0	0	0	0	0
.....	.....	0	30	0	Num.	—	∞	—	∞
.....	.....	0	0	0	0	0	0	0	2
.....	.....	0	63	0	Num.	—	∞	—	∞
.....	.....	0	0	0	1	10	7	Num.	—
.....	.....	0	0	0	2	0	8	0	Num.
.....	.....	0	0	0	1	2	2	7	11
—	∞	0	0	0	0	0	0	6	5
—	∞	0	0	0	0	0	Num.	—	Num.
.....	.....	0	5	0	33	0	Num	—	∞
.....	.....	0	Num.	—	Num.	—	∞	—	∞
.....	.....	1	0	5	0	28	6	Num.	—
.....	.....	0	0	1	0	1	0	Num.	—
.....	.....	0	2	3	7	0	22	—	Num.
—	Num.	.....	.....	.....	.....	.....	.....	.....	.....
3	Num.	4	0	14	4	Num.	Num.	—	∞
—	∞	0	Num.	—	∞	—	∞	—	∞
2	60	0	0	0	0	0	1	13	2
.....	.....	0	0	0	1	0	2	2	28
.....	.....	0	23	0	Num.	—	∞	—	∞
—	∞	.....	.....	0	1	0	1	0	3
.....	.....	0	4	0	15	0	Num.	—	∞
.....	.....	1	0	0	15	0	Num.	—	∞
.....	.....	0	3	0	Num.	0	Num.	—	∞
—	∞	.....	.....	.....	.....	.....	.....	.....	.....
—	∞	4	9	5	21	25	Num.	—	∞
—	∞	0	Num.	—	∞	—	∞	—	∞
—	∞	0	0	0	1	3	3	8	32
.....	.....	0	2	0	15	0	Num.	—	∞
.....	.....	1	1	0	0	0	5	7	13
.....	.....	0	15	0	Num.	—	Num.	—	∞

TABLE 6—*Continued*

RESULTS OF THE USE OF GLYCERIN AS PRESERVATIVE IN ROUTINE EXAMINATION OF TYPHOID STOOLS

Period of Delay and Percentage of Glycerin Used as a Preservative	Methylene-blue Eosin Plates					
	1:10,000		1:1000		1:100	
	Typhoid	Others	Typhoid	Others	Typhoid	Others
Patient A. M.						
X-4						
At once	.....	.....	.....	.....	.....	.....
24 hr. { 30% glycerin	.....	.....	.....	.....	.....	.....
24 hr. { Control stool	.....	.....	.....	.....	.....	.....
48 hr. { 30% glycerin	.....	.....	.....	.....	.....	.....
48 hr. { Control stool	.....	.....	.....	.....	.....	.....
3 da. { 30% glycerin	10	0	—	Num.	—	Num.
3 da. { Control stool	5	Num.	1	∞	—	∞
10 da. { 30% glycerin	.....	.....	.....	.....	.....	.....
10 da. { Control stool	.....	.....	.....	.....	.....	.....
15 da. { 30% glycerin	.....	.....	.....	.....	.....	.....
15 da. { Control stool	.....	.....	.....	.....	.....	.....
22 da. 30% glycerin	.....	.....	2	Num.	8	Num.
Patient E. O.						
X-11						
At once	.....	.....	.....	.....	.....	.....
24 hr. { 30% glycerin	0	1	2	0	3	8
24 hr. { Control stool	0	0	3	1	8	10
48 hr. { 30% glycerin	0	0	0	0	5	0
48 hr. { Control stool	0	1	0	0	0	10
4 da. { 30% glycerin	1	11	—	Num.	—	Num.
4 da. { Control stool	1	0	—	Num.	—	Num.
6 da. { 30% glycerin	0	0	0	0	0	0
6 da. { Control stool	0	0	0	0	0	0
15 da. 30% glycerin	.....	.....	0	Num.	—	∞
Patient M. B.						
X-11						
At once	.....	.....	.....	.....	.....	.....
24 hr. { 30% glycerin	10	16	14	81	Num.	—
24 hr. { Control stool	1	7	2	68	—	Num.
48 hr. { 30% glycerin	2	14	6	20	—	Num.
48 hr. { Control stool	0	6	1	45	8	Num.
Patient M. W.						
X-11						
At once	.....	.....	.....	.....	.....	.....
24 hr. { 30% glycerin	4	8	8	22	35	85
24 hr. { Control stool	0	65	0	Num.	—	∞
48 hr. { 30% glycerin	2	1	1	1	12	21
48 hr. { Control stool	0	Num.	0	Num.	—	∞
6 da. { 30% glycerin	.....	.....	.....	.....	.....	.....
6 da. { Control stool	.....	.....	.....	.....	.....	.....
15 da. 30% glycerin	.....	.....	.....	.....	.....	.....
Patient A. M.						
X-11						
At once	.....	.....	.....	.....	.....	.....
24 hr. { 30% glycerin	4	24	1	84	2	Num.
24 hr. { Control stool	0	60	0	Num.	—	∞
48 hr. { 30% glycerin	0	Num.	0	Num.	—	Num.
48 hr. { Control stool	0	Num.	0	Num.	—	∞
4 da. { 30% glycerin	0	Num.	0	Num.	—	Num.
4 da. { Control stool	0	Num.	0	—	—	∞

TABLE 6—*Continued*

RESULTS OF THE USE OF GLYCERIN AS PRESERVATIVE IN ROUTINE EXAMINATION OF TYPHOID STOOLS

Methylene-blue Eosin Plates		Brilliant-green Eosin Plates							
1:10		1:1000		1:100		1:10		1:1	
Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others	Typhoid	Others
.....	.....	0	0	0	0	0	2	8	10
.....	.....	0	0	0	0	3	8	—	Num.
.....	.....	0	0	1	0	10	3	29	2
.....	.....	0	0	0	0	0	2	25	35
.....	.....	0	30	0	Num.	—	∞	—	∞
—	Num.	0	0	0	0	0	3	15	Num.
—	∞	0	Num.	—	∞	—	∞	—	∞
.....	.....	0	0	1	0	2	0	28	0
.....	.....	0	11	2	53	12	Num.	—	∞
.....	.....	0	0	0	0	6	2	42	8
.....	.....	0	1	0	4	0	45	0	Num.
10	0	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	2	0	26	0	Num.	—
40	80	0	0	4	0	13	0	Num.	0
36	41	0	0	4	0	42	0	Num.	0
24	0	0	0	0	0	3	0	26	0
.....	.....	0	0	2	0	24	0	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
4	1	0	0	1	0	3	0	37	0
3	0	0	0	0	0	0	0	2	1
—	∞	.....	.....	0	0	0	3	0	Num.
.....	.....	.....	.....	2	0	51	0	Num.	1
—	∞	25	0	60	0	Num.	0	∞	—
—	∞	4	0	28	0	—	Num.	Num.	—
—	Num.	0	0	10	0	Num.	—	∞	2
—	∞	0	0	0	0	17	0	Num.	0
.....	.....	.....	.....	1	0	2	0	Num.	—
Num.	—	4	0	33	0	Num.	0	∞	0
—	∞	0	1	0	12	0	65	0	Num.
—	Num.	0	0	1	0	Num.	—	Num.	—
.....	.....	0	5	0	18	0	Num.	.....	.....
.....	.....	0	0	7	9	50	33	∞	—
.....	.....	0	34	0	66	0	Num.	—	∞
.....	.....	.....	.....	0	0	3	0	25	7
.....	.....	.....	.....	0	0	1	29	0	Num.
—	∞	7	0	33	0	Num.	0	Num.	Few
—	∞	0	0	0	1	2	9	0	Num.
—	∞	0	0	0	0	1	4	0	32
.....	.....	0	2	0	1	0	1	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

rubbed up in salt solution and filtered through cotton in order to remove the coarser particles. Such treatment of the stools would be impracticable in routine hospital work. We have therefore preserved a series of stools in the following manner.

Wide-mouthed, screw-topped bottles, such as are ordinarily used for sputum, were sterilized and filled about two-thirds full with 30% glycerin in 0.6% sodium-chlorid solution. The typhoid stool was added until the bottle was nearly full and a portion of the same stool was placed in a sterile empty bottle to serve as a control. If the stool was fluid, the glycerin mixture was merely shaken after the top had been screwed on the bottle; if the stool was solid, it was crushed in the 30% glycerin with a wooden spatula or glass rod, no attempt being made to break up thoroughly all the small lumps. At intervals of one to several days the glycerin mixture was shaken well, dilutions were prepared, and plates were inoculated as in the preceding experiments. At the same time a bit of the control stool was emulsified in salt solution to make a suspension of approximately the same density as that of the glycerin mixture. This suspension was diluted and inoculated on plates in a similar manner. Examples of stools treated in this way are shown in Table 6.

Table 6 shows that the proportion of typhoid to colon bacilli is not decreased in 30% glycerin for a period of a week or more, while there is an increase of B. coli in the control stool at the expense of the typhoid. Thus similar results are obtained when the stools are merely crushed in the 30% glycerin, and when they are thoroughly emulsified and the coarser particles removed by filtration through cotton.

When specimens are to be sent through the mail or by express, the feces should be added to 30% glycerin, not more than 1 part of feces to 2 parts of glycerin, in a bottle with a screw top such as is used for sputum. If the stool is solid, it should be broken up with a spatula or splinter of wood. The top to the bottle should be screwed down tightly and a bit of adhesive plaster wrapped around it to prevent its working loose. The bottle should then be shaken well, to distribute the stool material throughout the glycerin, and packed in double containers such as are used for mailing cultures. The 30% glycerin is prepared by adding glycerin to sterile 0.6% sodium-chlorid solution.

It is believed that the method just described will afford material aid in the stamping out of typhoid fever by making it easier to discover typhoid-carriers. The method should also assist the practicing physician in ascertaining, before he dismisses his typhoid-convalescent patients, whether or not they are still discharging typhoid bacilli with their stools.

With regard to the comparison of the three kinds of media used for the isolation of typhoid bacilli in these experiments, little need be said, since the facts are sufficiently obvious in the tables. It is seen that with almost every stool used large numbers of colonies of B. coli appear on the Endo and eosin methylene-blue plates, when the same material yields very few colon colonies on the eosin brilliant-green agar; it is seen that the typhoid organisms grow up as well on the latter medium as on the other two, the same material yielding practically the same number of typhoid colonies on the three different plates when only a few B. coli are present; it is seen finally that typhoid bacilli are time and again recovered on the eosin brilliant-green plate from material that yields negative results on the other two, while in no instance do the latter plates show typhoid colonies when the eosin brilliant-green plate is negative. It therefore follows that the eosin brilliant-green plate, if properly prepared, is far superior to the other two for the isolation of typhoid bacilli from stools.

The Endo and eosin methylene-blue plates yield similar results (see Table 4), but the typhoid colonies are more readily found on the latter, which is therefore to be preferred.